Problem 1

a) This is a simple Born solvation energy question, so we use our Born Equation for transfer of an ion. The dielectric constant of the water is ~80 and that of the interior of a membrane is ~2. The radii are given.

\[ \Delta G_{\text{transfer}}^0(D_1 \rightarrow D_2) = \frac{333 \cdot q^2}{2a} \left( \frac{1}{D_2} - \frac{1}{D_1} \right) \]

transfer energy for sodium 48 kcal/mol and 41.84 kcal/mol. Even though the sodium ion and the chloride ion are in the same period of the periodic table, the sodium ion lost the only electron it had in its outer shell, making it substantially smaller.

b) The potassium ion will be larger than the sodium ion because it is in the next period of the periodic system. Its transfer energy will, therefore, be smaller.

c) In response to a charge that is being placed into the protein interior, the “floppy” protein is better able to rearrange its internal dipoles (the peptide bonds in the interior are the most important dipoles in a protein) in response to the new charge. As a result, placing a charge inside the floppy protein should be energetically less costly. Obviously this question was aimed at the effects of flexibility on the dielectric constant of a protein. If we were doing this on a real protein, we then have to consider the possibility, that the floppy protein may have lower overall stability – floppiness and marginal stability often go hand-in-hand. The introduction of a charge may then be enough to cause the floppy protein to unfold. In which case the wild-type protein may be the better choice…

Problem 2

a) We simply write down the formula for the energy of coulombic interactions, set the energy to \(1RT = 0.6\ kcal/mol\) and solve for the distance \(r\).

\[ E^0_{\text{thermal}} = -kN_A T = -0.6 \text{kcal/mol} = \frac{333 \cdot q_1 \cdot q_2}{D \cdot r_{\text{bjerrum}}} \]

\[ r_{\text{bjerrum}} = \frac{333 \cdot q_1 \cdot q_2}{D \cdot 0.6} = \frac{333}{78.54 \cdot 0.6} = 7.06 \text{Å} \]

b) The formula for the coulombic interaction shows that if we increase the charge on both ions to two, the numerator increases fourfold. To keep the ratio of numerator and denominator fixed (this ratio is \(-0.6\ \text{kcal/mol}\)) we need to increase denominator by a factor of four as well.

So the \(r_{\text{bjerrum2}} = 29.4 \text{Å}\).

c) We could lower the temperature, thus dropping the thermal energy that masks the effect of the electrostatic interaction. To do so we would have to drop the temperature to somewhere around 75 deg K. This is well below freezing point, so that we would not have a solution anymore at all, but crystalline ice. So this does not work.
The other parameter we could adjust is the dielectric constant. By dropping D to 20 the singly charged ions will experience their mutual attraction/repulsion as strongly as doubly charged ions do in aqueous solution.

**Problem 3**

We do not have a formula to calculate the energy of transferring a saltbridge between media of differing dielectric constants, but we can break down the process into a series of steps that we can calculate using the Born and Coulomb formulas.

We first separate the two charges to infinity inside the high dielectric environment. This is a Coulomb calculation. Then we transfer the two charges separately (2 times the Born transfer energy). And finally we bring the charges back together, again a Coulomb calculation. These three steps get us to the same result as simply transferring the saltbridge and since the energy of a system is the state function (i.e. independent of the path we took to get to this state) the sum of the energies of the three steps will give us exactly the energy of transfer.
\[ \Delta G^0_{\text{transfer}} = -\Delta G^0_{\text{coulomb} D_1} + \Delta G^0_{\text{coulomb} D_2} + 2\Delta G^0_{\text{born} D_1 \rightarrow D_2} \]

\[ \Delta G^0_{\text{transfer}} = -\frac{333 \cdot q_1 \cdot q_2}{D_1 \cdot r} + \frac{333 \cdot q_1 \cdot q_2}{D_2 \cdot r} + 2 \frac{333 \cdot q^2}{2 \cdot a} \left( \frac{1}{D_2} - \frac{1}{D_1} \right) \]

with \( q_1 \cdot q_2 = -q^2 \)

\[ \Delta G^0_{\text{transfer}} = +\frac{333 \cdot q^2}{D_1 \cdot r} - \frac{333 \cdot q^2}{D_2 \cdot r} + 2 \frac{333 \cdot q^2}{2 \cdot a} \left( \frac{1}{D_2} - \frac{1}{D_1} \right) \]

\[ \Delta G^0_{\text{transfer}} = -\frac{333 \cdot q^2}{r} \left( \frac{1}{D_2} - \frac{1}{D_1} \right) + 2 \frac{333 \cdot q^2}{2 \cdot a} \left( \frac{1}{D_2} - \frac{1}{D_1} \right) \]

with \( r = 2a \)

\[ \Delta G^0_{\text{transfer}} = \frac{333 \cdot q^2}{2 \cdot a} \left( \frac{1}{D_2} - \frac{1}{D_1} \right) = \Delta G^0_{\text{born} D_1 \rightarrow D_2} \]

In other words the energy of burying a saltbridge is exactly the same as burying a single charge. This somewhat counterintuitive result makes sense if we think about the original picture we used to explain the Born energy. If we slowly charge up both ions, the charges on the portion of the two ions that touch one another are actually just as much attracted to the charges on the other ion than they are repelled by those charges that are building up on its own ion. So we do not have to put in any net energy to deliver those charges to the two spheres.

**Problem 4**

a)

b) Lysines are positively charged at neutral pH. It costs an enormous amount of energy to bury that charge inside the hydrophobic interior of a protein. This makes it very likely that lysine will be in the high-dielectric solvent. The lysine sidechain also has a large degree of conformational freedom. By exploring all its possible conformations Lysine increases its conformation entropy. The high degree of disorder seen for lysine is a direct reflection of the high degree of conformational entropy of the lysine sidechain. Tyrosines on the other hand have far fewer conformations that they can adopt, so less energy is needed to restrict their conformational freedom. The tyrosine’s phenolic oxygen-hydrogen bond is strongly polarized but the oxygen and hydrogen carry only a small partial charge. Burying these partial charges is far less energetic costly than burying a full
charge. At the same time the tyrosine sidechain can use its phenolic group to form hydrogen bonds, thus forming stabilizing interactions when it is exposed to water. So it is not surprising that tyrosine can be found both in the protein interior, due to its largely hydrophobic character but also stick its tip out into solvent because it can form hydrogen bonds.

c) The conformational free entropy of for this kind of problem is best calculated using the Boltzmann equation in its classic form

\[ S = k \ln W \]

and

\[ \Delta G_{\text{ordered} \rightarrow \text{disordered}}^0 = \Delta H^0 - T \cdot \Delta S^0 \]

with \( \Delta H^0 = 0 \)

\[ \Delta G_{\text{ordered} \rightarrow \text{disordered}}^0 = -T \cdot N_A \cdot (S_{\text{disordered}} - S_{\text{ordered}}) \]

simply plugging in the numbers (\( W_{\text{lys}} = 3 \times 3 \times 3 \times 3 = 81 \)) we get:

\[ \Delta G_{\text{lys ordered} \rightarrow \text{disordered}}^0 = -T \cdot N_A \cdot k \cdot (\ln W_{\text{disordered}} - \ln W_{\text{ordered}}) \]

\[ \Delta G_{\text{lys ordered} \rightarrow \text{disordered}}^0 = -0.6 \ \text{kcal/mol} \cdot 4.39 = -2.6 \ \text{kcal/mol} \]

and

\[ \Delta G_{\text{Tyr ordered} \rightarrow \text{disordered}}^0 = -T \cdot R \cdot (\ln 3 - \ln 1) \]

\[ \Delta G_{\text{Tyr ordered} \rightarrow \text{disordered}}^0 = -0.66 \ \text{kcal/mol} \]

The energy of ordering tyrosine is very similar to thermal energy (RT=0.6 kcal/mol at 298K) while that of lysine is a lot higher.

d) Due to the hydrophobic effect, the hydrophobic portion of the lysine side chain would prefer to be buried inside the protein interior away from the water. However, since the interiors of proteins are typically well packed, burying the sidechain would require us to restrain its conformational freedom—we just calculated how much energy this would cost. Burying the lysine would also require us to transfer a positively charged group with a radius of \( \approx 2.5 \ \text{Å} \) (\( r_{\text{nitrogen}}^+ r_{\text{hydrogen}} \)) from the high-dielectric of the solvent to the low dielectric of the protein interior.

So the total energy of burying a lysine sidechain, assuming that we would form hydrogen bonds both in the interior of the protein and with the solvent and that we therefore have no net contribution from hydrogen bonding, would be:
\[
\Delta G_{\text{burrying}}^0 = \Delta G_{\text{hydrophobic}}^0 + \Delta G_{\text{born}}^0 + \Delta G_{\text{conformational}}^0
\]
\[
\Delta G_{\text{burrying}}^0 = -4 \cdot 0.8 \text{kcal/mol} + \frac{333 \text{ kcal/(mol} \cdot \text{Å}) \cdot 1^2}{2 \cdot 2.5} \left( \frac{1}{D_{\text{protein}}} - \frac{1}{D_{\text{water}}} \right) + 2.6 \text{kcal/mol}
\]
\[
\Delta G_{\text{burrying}}^0 = -3.2 + 21.3 + 2.6 \text{kcal/mol} = 20.8 \text{kcal/mol}
\]

e) We get the equilibrium constant via

\[
\Delta G^0 = -RT \ln K_{eq}
\]
so

\[
K_{eq} = e^{-\frac{\Delta G^0}{RT}}
\]

\[
\frac{[\text{Lyss}_{\text{burried}}]}{[\text{Lyss}_{\text{solvent exposed}}]} = 9.3 \cdot 10^{-16}
\]

in other words the chance that we would ever find a charged lysine residue buried inside a protein would be extremely slim. So if we ever find one in a protein structure, it is probably a good bet that this buried lysine plays a central role in the proteins function.

**Problem 5**

a) Generating a zwitter ion is the equivalent of generating two ions of radius “a” at infinite distance (Born) and then bringing them together to the distance “r” (Coulomb). Note that we are not transferring the charges in solution, but we generate them in the interior of the protein.

\[
\Delta G_{\text{zwitterion}}^0 = 2\Delta G_{\text{Born}}^0 + \Delta G_{\text{Coulomb}}^0
\]
\[
\Delta G_{\text{zwitterion}}^0 = \frac{333 \cdot q^2}{2a} + \frac{333 \cdot q_1 \cdot q_2}{2r}
\]
\[\text{with } q_1 = -q_2\]
\[
\Delta G_{\text{zwitterion}}^0 = \frac{333 \cdot q^2}{2a} \left( \frac{1}{a} - \frac{1}{r} \right)
\]

b) Our equation has two terms. The Born term is destabilizing. Burying the charges costs energy. The Coulomb term is stabilizing. By moving the two oppositely charged ions from infinity to distance r, we get back some energy. Here is what we can do to minimize the overall energetic penalty of generating the zwitterion in the active site.

1) By increasing the dielectric constant we can lessen the energetic penalty of generating the two charges.
2) If we decrease r the stabilizing Coulomb term becomes larger. So if we place the two ions next to one another we pay a lower energy penalty. Note, we cannot make r shorter than 2a, so it will always cost us energy to generate the zwitter ion.

3) We could delocalize the charges. Making the radius “a” larger decreases the size of the Born term.

4) We could place a positive and a negative charge in the active site so that these charges are in contact with the charges of the zwitterion. As we saw in Problem 2 burying ion pairs costs just as much energy as burying individual ions. This allows us to pay the energetic cost of burying charges upfront, during the folding of the protein. Then during the catalytic cycle, generating the zwitterionic state will not cost any further energy, as each of the charges on the zwitterions is in contact with the compensating charges in the protein active site.